CYTOGENETIC INVESTIGATIONS OF ARTIFICIAL *HELIANTHUS GIGANTEUS* X *H. SALICIFOLIUS* HYBRIDS (COMPOSITAE)[1]

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Over the past several years biosystematic investigations of perennial species of *Helianthus* have resulted in the production of large numbers of artificial, interspecific hybrids (Long, 1955; Heiser et. al., 1962). Although analyses of these hybrids have been superficial in many instances, they have been adequate for purposes of determining approximate genetic relationships of the parent species. During a program of hybridization experiments with alternate-leaf perennial sunflowers, the artificial hybrid between *H. giganteus* L. and *H. salicifolius* A. Dietr. was secured. A detailed study of it has been made because this hybrid represents a cross between morphologically and ecologically widely divergent, allopatric species, and because of its striking resemblance to a third species, *H. grosseserratus* Martens. In addition to morphological and cytological comparisons, it has been possible to make observations on hybrid plants that were grown in two different experimental gardens and thus obtain information concerning the effects of en-

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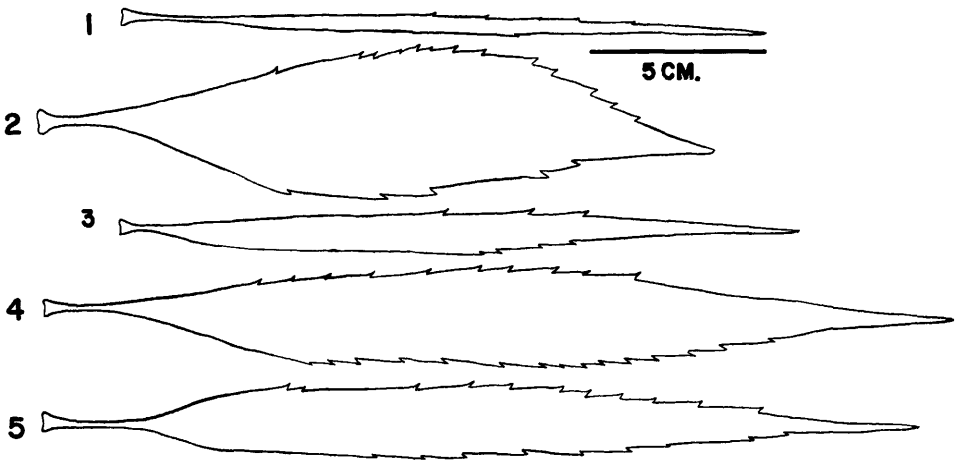
vironmental factors on morphological characters. The purpose of this investigation, therefore, has been three fold: (1) to examine the genetic and taxonomic relationship of the parent species; (2) to test the hypothesis that *H. grosseserratus* originated through natural hybridization of *H. giganteus* and *H. salicifolius*, and (3) to examine the effects of different environmental circumstances on those morphological characters used in distinguishing perennial species of *Helianthus*.

METHODS AND MATERIALS

Helianthus giganteus occurs in eastern United States, especially in the vicinity of the lower Great Lakes where it grows in swamps and other moist places. Its range is from North Carolina, north to Maine and Ontario and west to Minnesota. A highly variable species, it hybridizes with a number of other sunflowers, especially *H. grosseserratus* (Long, 1961). *Helianthus salicifolius* occurs in south-central United States where it grows in limestone glades and dry prairies, from Texas north into Kansas and Missouri. The F_1 hybrid was synthesized using a race of *H. giganteus* from Randolph Co., Indiana no. 97, and a race of *H. salicifolius*, no. 115, grown from seed supplied by the Pierce Seed Company (fig. 1–7). Methods



FIGURES 1 to 5. Typical cauline leaf outlines of parental plants and artificial hybrids. 1) *H. salicifolius*, no. 115; 2) *H. giganteus*, no. 97; 3) artificial hybrid *H. salicifolius* X *H. giganteus* 115 x 97; 4) segregrate backcross, *H. salicifolius–H. giganteus* X *H. giganteus*; 5) *H. grosseserratus*, no. 827, Pickaway Co. Ohio. Note close similarity in leaf morphology between figure 4 and 5. For further explanation see text.

used in this investigation for hybridizing plants, fertility tests, morphological comparisons, and cytological observations are the same as those described in previous reports (Long, 1959; 1960).

The two experimental gardens are located at Bloomington, Indiana, where biosystematic studies of *Helianthus* have been in progress for a number of years, and at Delaware, Ohio. The Bloomington garden is an open field of heavy clay soil. In 1956 transplants of the parent species and hybrid were made to the Delaware garden that, initially, was a shaded area of rich, black soil. Thus, contrasting environments were available for observing the effects of differences in soil, light, and to a lesser degree, temperature, rainfall, and other physical factors. In 1958 the site of the Delaware garden was moved to an open field comparable to the Bloomington garden, and here both parent species and hybrid approximated the Bloomington plants in vigor and size.

RESULTS

Morphological Observations on Hybrids

A summary of comparative observations and measurements of parent species and artificial hybrid is given in table 1. Data relative to the parent species were taken from plants grown in the Bloomington garden. Hybrid plants grown in the Bloomington garden in general resembled *H. giganteus*, but the transplant hybrid in the Delaware garden resembled *H. salicifolius*. Morphological characters of the hybrid strongly affected by the environmental differences were stem diameter, length of longest internode, stem color, absolute size of largest cauline leaf, length of phyllary and diameter of disk. No environmental modification is evi-

FIGURES 6–7. Photographs of garden cultures. 6) left, upper half of *H. giganteus* parent plant; right, upper half of *H. salicifolius* parent plant; 7) inflorescence and mid-cauline section of *H. salicifolius* X *H. giganteus* artificial hybrid.

dent in vesture of stem, leaf, and inflorescence, size of ligules, color and size of disk floret, and size of achenes. In annual sunflowers red or purple-brown disk floret color is apparently dominant to yellow (Cockerell, 1915a). In perennial sunflowers the reverse appears to be true.

Cytological Observations

The results of analysis of microsporogenesis are presented in table 2. Preliminary studies revealed that there was no difference in meiotic behavior of chromosomes in hybrids grown in different gardens and data for the hybrid given here are based on the Delaware plant. Although abnormalities occur during meiosis in hybrids, they are not as frequent as one might expect in a cross involving

distinctive *Helianthus* species. A rather high degree of chromosome homology obtains in the two plants (fig. 11). The most important kind of chromosomal abberration is found at diakinesis where approximately 25 per cent of cells examined contained rings or chains of four chromosomes. This would indicate the species differ structurally by at least one translocation. A large number of lagging chromosomes occur during metaphase I of hybrids suggesting disturbance of the timing mechanism of meiosis. As reduction-division proceeds, however, fewer

TABLE 1

Comparative morphology of artificial hybrids and parents

	H. giganteus	F_1 hybrid (Bloomington)	F_1 hybrid (Delaware)	*H. salicifolius*
Stem:				
diameter (cm)	2.2	2.1	1.4	1.7
vesture	hirsute	glabrous	glabrous	glabrous
internode (cm)	2.2–3.2	0.6–1.6	0.3–1.4	0.6–1.4
pigmentation	dark red-brown	light red-brown	yellow-green	yellow-green
Leaf:				
length (cm)	21.2–22.0	25.1–26.0	18.1–19.9	16.6–19.2
width (cm)	4.0–4.6	1.6–2.3	1.3–1.5	0.5–0.8
vesture	hirsute	puberulent	puberulent	puberulent
petiole length (cm)	1.6–2.3	0.7–0.8	1.3–2.2	0.0
petiole setae	18–21	none	none	none
Inflorescence:				
phyllary length (cm)	1.5–1.6	1.6–1.7	1.1–1.2	2.3–2.6
phyllary vesture	hirsute	puberulent	puberulent	glabrous
disk diameter (cm)	2.6–3.2	2.3–2.5	1.9–2.1	2.1–2.4
ray floret length (cm)	1.7	2.5–2.7	2.4–2.6	3.1–3.3
ligule width (cm)	0.5	0.8–0.9	0.7–1.0	0.8
ligule tip	obscurely bifid	obscurely bifid	obscurely bifid	strongly bifid
disk floret length (cm)	0.8–0.9	1.0–1.2	1.0	1.1
disk floret pigmentation	yellow	yellow	yellow	purple-brown
achene length (cm)	0.4	0.4–0.5	0.4–0.5	0.6–0.7

TABLE 2

Meiosis in artificial hybrid and parents

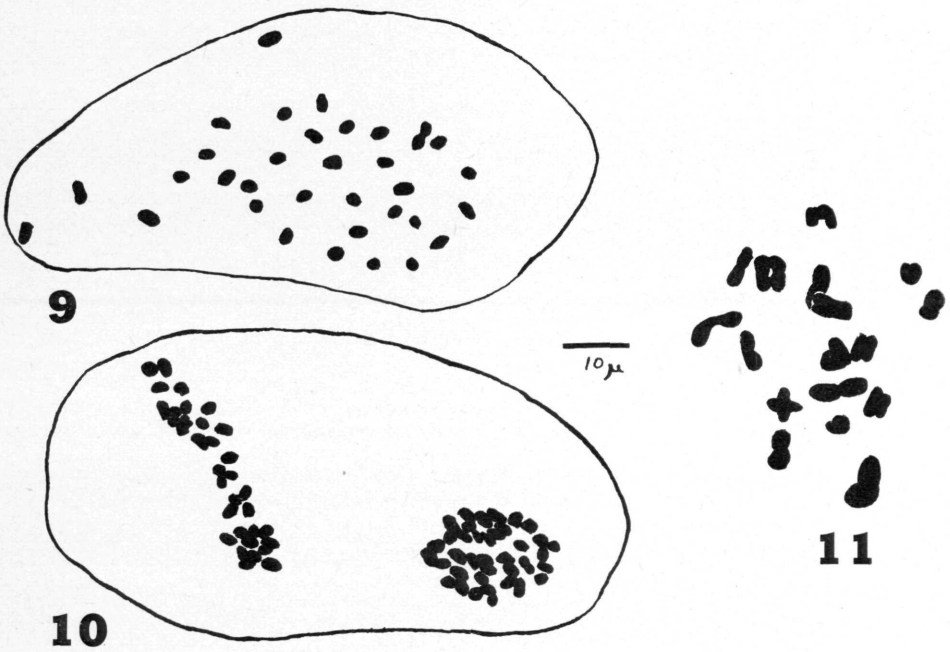
	Diakiensis				Metaphase I			Anaphase I				Telophase II		
	No.	17 II	Rings Chains	I's	No.	Normal	With Laggers	No.	Normal	Bridges	With Laggers	No.	Normal	Abnormal
H. giganteus	52	98.0	2.0	0.0	410	97.8	2.2	407	98.3	1.7	0.0	403	100.0	0.0
F_1 hybrid	54	76.0	24.0	0.0	403	89.6	10.4	406	96.0	0.5	3.5	409	99.8	0.2
H. salicifolius	54	85.2	9.3	5.5	406	96.1	3.9	401	94.6	2.7	2.7	405	99.4	0.6

abnormalities are apparent. It should be noted that bridge-fragment and chromatid-bridge formation during anaphase I is slightly higher in parent species than in hybrids. Bridges at anaphase were frequently double and without any fragments observed, similar to those reported for *H. grosseserratus* X *H. salicifolius* hybrids (Long, 1957).

Somatic chromosome numbers were obtained from root-tip smears of the

Delaware F_1 hybrid (fig. 9, 10). The chromosome number was $2n=34$ as was expected. Some variation in chromosome morphology was observed. The chromosomes range in size from 3.2 to 6.5 μ with the mean 4.5 μ. The significance of karyotype in *Helianthus* taxonomy has not been demonstrated, however.

FIGURE 8. Photograph of garden cultures. Heads of mutant backcross *H. salicifolius–H. giganteus* X *H. giganteus* showing twisted ray florets and cupped ligules. Background scale each division equals 1 dm.



FIGURES 9 to 11. Camera lucida drawings of chromosomes of *H. salicifolius* X *H. giganteus* hybrid. 9) somatic chromosomes, late prophase, from root-tip preparation, $2n=34$; 10) somatic chromosomes, telophase; 11) meiotic chromosomes, normal diakinesis, $n=17$, from pollen mother cells. For further explanation see text.

Fertility of Parent Species and Hybrids

Relative fertility of parent plants and hybrids was measured both by differential staining reaction of pollen in cotton blue and by seed set. Pollen fertility of *H. giganteus* and *H. salicifolius* plants was high, ranging from 93.0 to 96.0 per cent stainable pollen. Seed-set in intra-specific crosses in *H. giganteus* averages 84 per cent normal achenes, 16 per cent aborted achenes, and no undeveloped ovaries (Long, 1960). The F_1 hybrid was 70.5 per cent pollen fertile, and in crosses of *H. giganteus* X *H. salicifolius* average seed-set was 20.1 per cent normal achenes, 14.4 per cent aborted achenes, and 65.5 per cent undeveloped ovaries. In backcrosses of the F_1 hybrid to *H. giganteus* as the recurrent parent, pollen fertility was 81.3 per cent, and seed-set 28.6 per cent normal achenes, 20.0 per cent aborted achenes, and 51.4 per cent undeveloped ovaries. Backcross plants, therefore, regained considerable fertility.

F_1 hybrid (H. giganteus x H. salicifolius) *backcrosses*

Hybridization of the F_1 *H. giganteus* X *H. salicifolius* with *H. giganteus* no. 864 from Fulton Co., Ohio, invariably produced a mutant plant that bore heads that failed to open completely. The comparative morphology of this hybrid is given in table 3. The plant has been growing in the experimental garden at Delaware for 4 years and, although producing only one or two stems each summer, appears to be vigorous otherwise. The heads have cupped, slightly twisted rays that enclose the disk (fig. 8). In many respects this compares closely to various mutant forms listed for annual sunflowers by Cockerell (1915b). Dissection of the disk florets revealed small undeveloped ovaries but normal appearing stamens. Apparently good pollen is produced with approximately 46.5 to 85.3 per cent stainability. Some heads, especially those produced later, aborted entirely with disk undeveloped and necrotic. The plant is functionally sterile because even those florets that produce good pollen remain enclosed in the chaff and the corolla tubes fail to open. The ray florets produce a variable number of awns. Ordinarily 2 awns (pappus) are present and they are thought to be highly modified sepals. But in the hybrid ray florets with 4 awns were most common, these 1.0 to 1.5 mm by 0.5 mm broad and somewhat leaf-like. Ray florets with 1,2, 3, and 5 awns were also observed. Backcrosses with 7 races of *H. giganteus* other than no. 864 produced normal, fertile, but highly variable hybrids. These plants could be classified as somewhat narrow-leaf *H. grosseserratus*.

Because of the morphological similarity of the hybrid to *H. grosseserratus*, it was of special interest to test the interfertility of these two plants. Six different cultures of *H. grosseserratus* were available for hybridization experiments, all obtained from Ohio, and the results of crosses were very similar to backcrosses of the hybrid with *H. giganteus* (table 3).

DISCUSSION

Cytogenetic investigations in *Helianthus* have been fruitful in establishing species-relationships and clarifying the taxonomy of difficult groups. This report clearly demonstrates that *H. giganteus* and *H. salicifolius*, although distinct morphologically and of diverse geographic origins, are elements of the same species-complex. The F_1 hybrid is fertile and vigorous, and it can be successfully backcrossed to either parent. Experimental introgression can thus be effected in the garden and an array of highly variable plants produced that equals the parent-species in vigor and fertility. These results were anticipated since earlier investigations showed that *H. grosseserratus* and *H. salicifolius* could be crossed (Long, 1955b) and *H. giganteus* easily hybridizes with *H. grosseserratus* (Long, 1960). Fertility of hybrids would indicate, however, that *H. salicifolius* is not so closely related to *H. giganteus* as is *H. grosseserratus*. Cytological observations during microsporogenesis, in which a higher incidence of chromosomal abberration was

noted in *H. giganteus* X *H. salicifolius* hybrids, confirm this conclusion. Also, the production of a sterile, mutant backcross indicates that some genetic barriers to cross-breeding exist.

Perhaps the most interesting aspect of this investigation has been the examination of the possibility that *H. grosseserratus* arose from hybridization of *H. giganteus* and *H. salicifolius*. Unlike comparable studies in annual species of *Helianthus*, thus far it has not been possible to determine much regarding the probable evolution of perennial species. Heiser and Smith (1960) adduced evidence for considering the cultivated ornamental sunflower *H. multiflorus* L. as having derived from hybridization of *H. annuus* and *H. decapetalus*. Long (1955b) demonstrated the probability that *H. kellermanii* Britton was the result of hybridization between *H. grosseserratus* and *H. salicifolius*. *Helianthus doronicoides* Lam. was shown to be a hybrid of *H. mollis* and *H. giganteus* (Jackson, 1956). When a detailed comparison is made between *H. giganteus* X *H. salicifolius* F_1 hybrids and *H. grosseserratus* plants, similarities are easily seen (tables 1 and 3). They are particularly notice-

TABLE 3

Comparative morphology of H. giganteus *X* H. salicifolius *backcrosses*

	H. giganteus-salicifolius X *H. giganteus* (mutant form)	*H. giganteus-salicifolius* X *H. grosseserratus*	*H. grosseserratus*
Stem:			
diameter (cm)	0.73	1.22	1.14
vesture	puberulent	puberulent, glaucous	glabrous, glaucous
internode (cm)	1.9–3.5	0.4–1.8	2.8–4.2
pigmentation	dark red-brown	red-brown	green-yellow
Leaf:			
length (cm)	13.8–14.5	21.5–22.4	21.4–29.5
width (cm)	1.3–1.5	1.8–3.2	4.0–4.6
vesture	puberulent	puberulent	hirsute above, tomentose below
petiole length (cm)	0.9	3.2–3.8	4.0–4.8
petiole setae	4.6	none	none
Inflorescence:			
phyllary length	1.2	1.2	1.1–1.2
phyllary vesture	puberulent	puberulent	glabrous
diameter of disk (cm)	0.63–0.92	1.27–2.00	1.50–2.27
ray floret length (cm)	1.9	1.8–2.0	2.1
ligule width (cm)	0.9	0.5	0.5
ligule tip	truncate	obscurely bifid	obscurely bifid
disk floret length (cm)	0.3	0.8	0.6
disk floret pigmentation	yellow	yellow	yellow
achene length (cm)	0	0.5	0.4

able in vesture and pigmentation of stem, length of leaf, absence of petiolar setae, length of ray floret, vesture and general morphology of phyllaries, and size of achene. Differences are primarily those of leaf-width and petiole length (fig. 1–5). Additional evidence for believing *H. grosseserratus* of recent and possibly hybrid origin comes from analyses of natural populations in Illinois and Ohio. Studies showed that not only was there considerable morphological variation, especially in leaf and phyllary characteristics, but a rather high degree of abnormal chromosomal behavior obtained (Long, 1961). Finally, it should be noted that the center of distribution of *H. grosseserratus* falls between those of *H. giganteus* and *H. salicifolius*. Judging from present distribution, *H. giganteus* apparently is centered in the ancient Appalachian Highlands and has migrated into the Great Lakes region since the retreat of the last glacier. *Helianthus salicifolius* is generally

confined to the vicinity of the very old Ozark Plateau. *Helianthus grosseserratus* chiefly occupies soils that have been available to plants only since Pleistocene times although it is known to occur sporadically in geologically older areas. Thus, an hypothesis can be advanced that considers *H. grossesserratus* a relatively new, rapidly evolving species that has arisen through natural hybridization of *H. giganteus* and *H. salicifolius* in some former time when these species were at least partially sympatric, perhaps in pre-Pleistocene times. A period of geographical isolation for the "new" species would be required in order to account for the occurrence of mutations that have led to some morphological divergence. Given these circumstances regarding origin, it is not surprising that *H. giganteus* and *H. grosseserratus* are highly interfertile nor that hybridization occurs in nature when *H. salicifolius* and *H. grossesserratus* come together.

Heiser (1961) has commented on the difficulty of documenting the origin of species through hybridization at the diploid level in *Helianthus*. He correctly points out that for every species for which we might suggest a hybrid origin, one might also postulate an origin through geographical speciation. Thus, *H. grossesserratus* might have derived from *H. giganteus* or *H. salicifolius* or a common ancestral form of both, through geographical isolation followed by mutations. The weight of circumstantial evidence, however, favors a hybrid-origin for *H. grosseserratus* because of its morphological similarity to the artificial hybrid, its geographical range, ecological preferences, and high interfertility with the artificial hybrid and parent species.

The observations on transplants of hybrid plants in different gardens provided information useful in biosystematic revisions of *Helianthus*. In perennial species the most valuable characteristics, taxonomically, appear to be vesture of stem and leaf, and dimensions of ligules, disk florets and achenes. These observations cast some doubt on the validity of taxonomic investigations in perennial *Helianthus* that are based on absolute size of leaves, disk diameter, phyllary length or internode patterns. It is unfortunately true, however, that unless these morphological characters are used, the identification of sunflower species as they are presently understood is very difficult. This may be a strong argument for supporting a considerably broader species concept in perennial *Helianthus* than has existed in recent years.

SUMMARY

The artificial hybrid *Helianthus giganteus* X *H. salicifolius* represents a successful cross of two morphologically dissimilar sunflowers of diverse geographical origins. A detailed morphological and cytological investigation was made to determine cytogenetic relationships of the parent species. Fertile, vigorous backcross plants were produced except for the sterile mutants produced when one race of *H. giganteus* was used as the recurrent parent. Evidence supports the conclusion that *H. salicifolius* belongs in the *H. giganteus* species-complex.

Because the hybrid closely resembles a third species, *H. grosseserratus*, special consideration was given to the possibility that this species arose through natural hybridization and represents a relatively "new" species closely allied to its parents.

Observations of hybrid plants in different experimental gardens provided information concerning the effects of environmental factors on commonly used taxonomic characters.

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